First report of a bla_{VIM-1} metallo-β-lactamase-possessing Klebsiella michiganensis

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HIGHLIGHTS

- We characterized the first *bla*$_{VIM}$-possessing *K. michiganensis* isolate (BD-50-Km)
- The strain was isolated from the rectal swab of a Turkish patient in Switzerland
- *bla*$_{VIM-1}$ was inserted in a rare class 1 integron harbored by a novel IncC plasmid
- Core-genome analysis showed that BD-50-Km was not closely-related to other *K. michiganensis* deposited in NCBI
- The backbone of the *bla*$_{VIM-1}$-carrying plasmid was common to other IncC plasmids, but its resistance region was unique

First report of a *bla*$_{\text{VIM-1}}$ metallo-β-lactamase-possessing *Klebsiella michiganensis*

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Running title: First VIM-1-positive *K. michiganensis*

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ABSTRACT

Background. *Klebsiella michiganensis* is an emerging pathogen. As for *Klebsiella pneumoniae*, this species is able to acquire antibiotic resistance genes (ARGs) via mobile genetic elements. In this context, *K. michiganensis* isolates producing carbapenemases of KPC, NDM, IMP and OXA-48-like types have been already reported. Here, we characterized a strain (BD-50-Km) isolated from the rectal swab of a Turkish patient hospitalized in Switzerland.

Methods. Species identification was initially obtained by using the MALDI-TOF MS. Susceptibility tests were done by the microdilution method. Whole-genome sequencing (WGS) was performed with both Illumina and Nanopore platforms and used to confirm ID, characterize plasmids and perform core-genome analyses.

Results. BD-50-Km was initially identified as *Klebsiella oxytoca* and showed a reduced susceptibility to imipenem. However, WGS indicated that the isolate was actually *K. michiganensis.* BD-50-Km carried the $bla_{\text{VIM-1}}$ associated to a rare class 1 integron (In*87*) located in a pST1 196kb IncC plasmid. This plasmid shared its backbone with many other IncC plasmids found in different species (including 5 *K. michiganensis*), but not the same In*87* and the remaining region harboring various ARGs. BD-50-Km belonged to the novel ST342. Moreover, core-genome analysis (single nucleotide variants analysis) showed that BD-50-Km was not closely-related to any of the *K. michiganensis* strains deposited in NCBI (n=212), including the 38 so far reported as possessing carbapenemase genes.

Conclusions. This is the first report of a bla_{VIM}-possessing *K. michiganensis* clinical isolate. The spread of plasmid-mediated VIM carbapenemases in this emerging pathogen represent an additional threat to our therapeutic armamentarium.

KEY WORDS: *Klebsiella*, carbapenemases, VIM, MBL, plasmid, integron

1. INTRODUCTION

Klebsiella michiganensis is emerging as an important human pathogen. Since its first report from a toothbrush holder in 2013, *K. michiganensis* isolates have been associated to nosocomial infections [1, 2]. For instance, an ESBL producer was responsible for an outbreak in a neonatal unit in Queensland (Australia) in 2018 [3], NDM-1 carbapenemase producers - that may also co-produce OXA-181 - were isolated from patients in South Africa during 2012-2013 [4, 5], and a strain simultaneously producing KPC-2, NDM-1, NDM-5 carbapenemases was detected in a patient with acute diarrhoea in 2016 in China [2]. With regard to Europe, the first KPC-3-producing *K. michiganensis* strain from a clinical patient was isolated in 2017 in Switzerland [6].

Overall, the emergence of carbapenemase producers in this species represents a serious threat, especially when the encoding *bla* genes possess the ability to disseminate horizontally via mobile genetic elements (MGEs) among different bacterial hosts. This is the case of the class B metallo-β-lactamases of the VIM family that have been described globally in many species of Enterobacterales [7]. Nevertheless, to our best knowledge, VIM-producing *K. michiganensis* strains have never been described.

In the present work, we characterized the first *K. michiganensis* strain possessing a multi-drug resistance plasmid encoding the $bla_{\text{VIM-1}}$ carbapenemase gene isolated from a patient hospitalized in Switzerland.

2. MATERIALS AND METHODS

2.1 Isolation, identification and antimicrobial susceptibility tests (ASTs)

On January 2018, a 59-years-old Turkish female who suffered from liver cirrhosis came to Switzerland for consultation and rehabilitation. No further clinical data are available. At admission, the patient underwent rectal swab to screen for multidrug-resistant (MDR) Gram-negatives. Both ChromID® ESBL and CARBA SMART selective agar plates (bioMérieux) were inoculated and incubated overnight at 35-36°C.

Species identification was routinely obtained using the matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS; Bruker); it was then achieved using whole-genome sequencing (WGS) data and the implementation of the Type (Strain) Genome Server (https://tygs.dsmz.de/), which uses a genome-based phylogeny approach, together with, for example, digital DNA:DNA hybridization (dDDH) and confidence interval support values to infer accurate species identification. ASTs were performed using the broth microdilution ESBF and GNX2F Sensititre panels (Thermo-Fisher). Minimum inhibitory concentrations (MICs) for antibiotics were interpreted according to the 2019 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (https://www.eucast.org/; version 9.0). The new CT103XL microarray (Check-Points) was implemented to screen for the presence of the main carbapenemase and ESBL *bla* genes [8].

2.2 Whole-genome sequencing (WGS)

WGS was performed using both NovaSeq 6000 (Illumina) and MinION (SQK-RBK004 library; FLO-MIN 106D R9 flow-cell; Oxford Nanopore) sequencing technologies as previously described [9]. In brief, the sequencing adapters from both Illumina and Nanopore reads were removed using Trimmomatic (v0.36) and Porechop (v0.2.4) with default parameters, respectively. A subset of best quality Nanopore reads were extracted with Filtlong (v0.2.0) using the Phred quality scores from the FASTQ file. The long-read assembly was

generated with Canu (v2.1), with approximate genome size set to 6.2 Mb. The subsequent assembly was circularized with Circlator (v1.5.5), using Canu as the assembler, and manually revised for errors with Nucmer (v3.1). The hybrid assembly was generated by multiple rounds of read correction and alignment with Illumina reads using Pilon (v1.22) and Bowtie2 (v2.3.4.1). Annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline, and insertion sequences (ISs) were manually curated with ISfinder (https://isfinder.biotoul.fr/). The final genome was analyzed using the tools of the CGE (www.genomicepidemiology.org/). MLST (v2.0) was performed according to the *K. oxytoca* scheme. Virulence factors were screened with the vfdb database (accessed on 30.11.2020) using ABRicate (v1.0.1), and metal resistance genes with AMRfinderPlus (v3.8.4). Polysaccharide locus analysis was conducted with the Kaptive webtool (https://kaptive-web.erc.monash.edu/).

The *bla*$_{\text{VIM-1}}$-harbouring integron was classified according to INTEGRALL (http://integrall.bio.ua.pt/). The complete genome assembly of BD-50-Km is deposited in GenBank (CP061930-CP061933) under BioProject PRJNA664790.

2.3 Core-genome analysis

The assemblies used for the core-genome analysis were retrieved from the NCBI isolate browser using the following queries: "scientific_name: *Klebsiella michiganensis*" to retrieve all available assemblies (n=212 *K. michiganensis*) and "scientific_name: AND AMR_genotypes: *bla*$_{\text{IMP}}$* *bla*$_{\text{KPC}}$* *bla*$_{\text{NDM}}$* *bla*$_{\text{OXA}}$*" as determined by the unique carbapenem genes present in "subclass: CARBAPENEM" in the MicroBIGG-E browser (n=38) (access/download date: 23.10.2020).

To look for other sequence type (ST) 342 strains, the full dataset (n=212) was subjected to an in-silico analysis with a custom script using the forward and reverse sequence alleles of ST342 (*gapA*_3, *infB*_5, *mdh*_40, *pgi*_64, *phoE*_24, *rpoB*_6, *tonB*_82) available at PubMLST

(pubmlst.org/organisms/klebsiella-oxytoca). Closely-related strains were detected by a global core-genome alignment of all available 212 *K. michiganensis* strains (data not shown). The tree was visualized with iTOL (v5.6.3; itol.embl.de).

3. RESULTS AND DISCUSSION

3.1 Phenotype and screening for carbapenemase genes

From the selective plates, a *Klebsiella oxytoca* strain (BD-50-Km) was identified by using the MALDI-TOF MS. Nevertheless, based on WGS, BD-50-Km was determined to belong to the *Klebsiella michiganensis* species. As shown in Table 1, the strain was resistant to aminoglycosides, β-lactam/β-lactamase combinations and last-generation cephalosporins, and also showed a slightly reduced susceptibility to carbapenems (e.g., MICs for imipenem and meropenem: 2 and <1 mg/L, respectively). According to the new CT103XL microarray, BD-50-Km was a bla_{VIM}, $bla_{\text{SHV-1-like}}$, and $bla_{\text{TEM-1-like}}$ carrier (data not shown).

3.2 Antimicrobial resistance genes (ARGs), serotype, and virulence factors (VFs)

BD-50-Km was composed of a chromosome (6.3 Mb) and carried 3 plasmids: pBD-50-Km_VIM-1 (196.3 kb, IncC (pST1)), pBD-50-Km_2 (202.3 kb, IncR), and pBD-50-Km_3 (135.6 kb, IncFII(K)). The following ARGs were detected: chromosome ($bla_{\text{OXY-1-7}}$, *aph(3')-Ia*), and plasmid pBD-50-Km_VIM-1 ($bla_{\text{VIM-1}}$, $bla_{\text{OXA-10}}$, $bla_{\text{SHV-2}}$, $bla_{\text{TEM-1B}}$, *aac(3)-IId, aac(6')-IIc, aadA1, ant(2'')-Ia, aph(3'')-Ib, aph(6)-Id, arr-2, cmlA1, mph(A), qnrA1, sul1*, Δ*sul2*).

The $bla_{\text{VIM-1}}$ promoter region was analyzed showing a strong P1 [-35: TTGACA; -10: TAAACT] and an inactivated P2 promoter (data not shown). This genetic background could not explain the low resistance expressed by BD-50-Km against carbapenems. However, we note that a consistent susceptibility pattern for carbapenems was observed for *E. cloacae* and *K. pneumoniae* isolates carrying the same $bla_{\text{VIM-1}}$ integron cassette as ours (see below) [10, 11].

Lipopolysaccharide (LPS; O), capsular (K) serotypes, and VFs of *K. pneumoniae* have been extensively characterized in the past. For instance, K1/K2 serotypes are linked to hypervirulence, and yersiniabactin producers are highly prevalent and frequently associated to

respiratory infections [12, 13]. In contrast, little is known about the serotypes and VFs of *K. michiganensis*. In this context, BD-50-Km carried novel K and O serotype variants, with a closest match to K locus KL161 and O locus O1v1, respectively (data not shown). Similarly, BD-50-Km was positive for multiple VF homologues, for instance, coding for yersiniabactin, enterobactin, type VI secretion system, heat-stable enterotoxin, capsule, type 1 fimbriae, and others (full results in File S1). Our strain also carried metal resistance genes for silver/copper (*sil* and *pco* gene families) in pBD-50-Km_2, and for mercury resistance (*mer* operon), present in pBD-50-Km_VIM-1. Heavy metal resistance, such as the *mer* operon, has been reported in multiple species and is an important mercury-detoxification system in contaminated environments [14].

3.3. Clonality and core-genome analyses

BD-50-Km carried two novel alleles in the *pgi* and *tonB* loci, and thus was assigned to the new ST342. To look for other strains similar to BD-50-Km and to understand the current prevalence of carbapenemase genes in *K. michiganensis*, we conducted a database search of *K. michiganensis* genomes with the NCBI isolate browser (www.ncbi.nlm.nih.gov/pathogens/isolates/).

Our search resulted in 212 available genomes of which 38 possessed carbapenemase genes of IMP-, KPC-, NDM-types, and OXA-181. A core-genome alignment of the 38 genomes along with 3 non-carbapenemase producers and BD-50-Km was constructed with Parsnp (v1.2) as previously described, and resulted in 178’921 single nucleotide variants (SNVs) [9]. Our isolate belonged to a group of *K. michiganensis* strains of international origin and of various STs. In particular it was distantly related to a ST27 cluster containing the Swiss KPC-3 producer strain KMISG1 (18’784 different SNVs) [6], and to two non-carbapenemase producers of ST213 from the USA and UK (18’638 different SNVs) (Figure 1). Our analysis confirmed that BD-50-Km is unique and does not belong to a clonal lineage.

3.4 bla$_{VIM-1}$-carrying plasmid characterization

An analysis of the above 42 WGS genomes with PlasmidFinder (v2.1) detected multiple replicon sequences per genome (data not shown). Of these, 5 were positive for an IncC replicon sequence of similar backbone to pBD-50-Km_VIM-1, but all were *bla*$_{VIM}$-negative (Figure S1).

Our *bla*$_{VIM-1}$-containing plasmid pBD-50-Km_VIM-1 was most similar to pVb0267 (MF627444) from *V. parahaemolyticus* isolated from shrimp in China. However, it also shared a similar backbone to other IncC plasmids, such as *Shewanella algae* p18064-65-CSB-B isolated from chicken stools in Tanzania [15], and to various multi-host *bla*$_{VIM-1}$-possessing strains (Figure 2 and Figure S2).

The *bla*$_{VIM-1}$ of pBD-50-Km_VIM-1 was associated to a small class 1 integron (In*87*) from *Enterobacter cloacae* (AY648125), first described in 2003 in Greece and also carrying the aminoglycoside-modifying enzyme *aac(6')-IIc* [10]. In*87*, including its recombination site *attI* (bp positions: 66213-66270), was inserted in between flanking IS elements, IS*4321* and Tn*3* family/IS*5075*. The same integron was responsible for an outbreak of *K. pneumoniae* bloodstream infections in Italy in 2004-2005 [11]. In Greece, other VIM producers have also been responsible for outbreaks [16-18]; moreover, the same In*87* has also been detected in four *Enterobacter hormaechei* [7, 19], though none of them was associated to IncC plasmids (Figure S3). Likewise, of the other *bla*$_{VIM-1}$-positive IncC plasmids identified, none of them carried the same *bla*$_{VIM-1}$-containing integron cassette as pBD-50-Km_VIM-1, highlighting its rarity (Figure S4).

The remaining ARGs of pBD-50-Km_VIM-1 were carried in a complex region containing multiple copies of various IS elements such as IS*5075*, IS*4321*, and IS*26*. Notably, *bla*$_{SHV-2}$ was associated to an IS*26* composite transposon also found in plasmid pTC2 from *Providencia stuartii*; and upstream, another class 1 integron carrying *ant(2")-Ia*, *arr-2*,

cmIA1, *bla*$_{\text{OXA-10}}$, and *aadA1*, present also in pVb0267, sharing a similar backbone to ours (Figure 2).

4. CONCLUSIONS

This is the first report of a *bla*$_{\text{VIM-1}}$-positive *K. michiganensis* clinical isolate (BD-50-Km). The strain harbored a novel IncC plasmid carrying *bla*$_{\text{VIM-1}}$ associated to a rare integron. Core-genome analysis showed that BD-50-Km did not belong to a clonal group. However, since the strain was isolated from a Turkish patient, it is likely that BD-50-Km originated from that region, where VIM producers have been frequently reported, such as in *Klebsiella* spp. and *Enterobacter* spp. [19, 20].

Since its discovery, *K. michiganensis* has proven to become an important human emerging pathogen. However, since this species is frequently misidentified as *K. oxytoca* by using the MALDI-TOF MS [2, 3, 6], information regarding its clonality, plasmid(s) background, serotype, and virulence factors is still limited. Therefore, it is imperative to perform more surveys to understand the role of *K. michiganensis* in human infections and its potential to acquire life-threatening MDR genes.

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ETHICAL APPROVAL: Not required

COMPETING INTERESTS: None to declare

REFERENCES

[1] Saha R, Farrance CE, Verghese B, Hong S, Donofrio RS. *Klebsiella michiganensis* sp. nov., a new bacterium isolated from a tooth brush holder. Curr Microbiol. 2013;66:72-8.

[2] Zheng B, Xu H, Yu X, Lv T, Jiang X, Cheng H, et al. Identification and genomic characterization of a KPC-2-, NDM-1- and NDM-5-producing *Klebsiella michiganensis* isolate. J Antimicrob Chemother. 2018;73:536-8.

[3] Chapman P, Forde BM, Roberts LW, Bergh H, Vesey D, Jennison AV, et al. Genomic Investigation Reveals Contaminated Detergent as the Source of an Extended-Spectrum-β-Lactamase-Producing *Klebsiella michiganensis* Outbreak in a Neonatal Unit. J Clin Microbiol. 2020;58.

[4] Founou RC, Founou LL, Allam M, Ismail A, Essack SY. Genomic characterisation of *Klebsiella michiganensis* co-producing OXA-181 and NDM-1 carbapenemases isolated from a cancer patient in uMgungundlovu District, KwaZulu-Natal Province, South Africa. S Afr Med J. 2018;109:7-8.

[5] Pedersen T, Sekyere JO, Govinden U, Moodley K, Sivertsen A, Samuelsen O, et al. Spread of Plasmid-Encoded NDM-1 and GES-5 Carbapenemases among Extensively Drug-Resistant and Pandrug-Resistant Clinical Enterobacteriaceae in Durban, South Africa. Antimicrob Agents Chemother. 2018;62.

[6] Seiffert SN, Wuthrich D, Gerth Y, Egli A, Kohler P, Nolte O. First clinical case of KPC-3-producing *Klebsiella michiganensis* in Europe. New Microbes New Infect. 2019;29:100516.

[7] Matsumura Y, Peirano G, Devinney R, Bradford PA, Motyl MR, Adams MD, et al. Genomic epidemiology of global VIM-producing Enterobacteriaceae. J Antimicrob Chemother. 2017;72:2249-58.

[8] Bernasconi OJ, Principe L, Tinguely R, Karczmarek A, Perreten V, Luzzaro F, et al. Evaluation of a New Commercial Microarray Platform for the Simultaneous Detection of β-Lactamase and *mcr-1* and *mcr-2* Genes in Enterobacteriaceae. J Clin Microbiol. 2017;55:3138-41.

[9] Campos-Madueno EI, Bernasconi OJ, Moser AI, Keller PM, Luzzaro F, Maffioli C, et al. Rapid Increase of CTX-M-Producing *Shigella sonnei* Isolates in Switzerland Due to Spread of Common Plasmids and International Clones. Antimicrob Agents Chemother. 2020;64.

[10] Galani I, Souli M, Chryssouli Z, Orlandou K, Giamarellou H. Characterization of a new integron containing bla_{VIM-1} and *aac(6')-IIc* in an *Enterobacter cloacae* clinical isolate from Greece. J Antimicrob Chemother. 2005;55:634-8.

[11] Cagnacci S, Gualco L, Roveta S, Mannelli S, Borgianni L, Docquier JD, et al. Bloodstream infections caused by multidrug-resistant *Klebsiella pneumoniae* producing the carbapenem-hydrolysing VIM-1 metallo-β-lactamase: first Italian outbreak. J Antimicrob Chemother. 2008;61:296-300.

[12] Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol. 2020;18:344-59.

[13] Russo TA, Marr CM. Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev. 2019;32.

[14] Boyd ES, Barkay T. The mercury resistance operon: from an origin in a geothermal environment to an efficient detoxification machine. Front Microbiol. 2012;3:349.

[15] Endimiani A, Bernasconi OJ, Budel T, Campos-Madueno EI, Kuenzli E, Hatz C, et al. Whole-Genome Characterization of a *Shewanella algae* Strain Coharboring $bla_{CTX-M-15}$ and *armA* Genes on a Novel IncC Plasmid. Antimicrob Agents Chemother. 2020;64.

[16] Vatopoulos A. High rates of metallo-beta-lactamase-producing *Klebsiella pneumoniae* in Greece--a review of the current evidence. Euro Surveill. 2008;13.

[17] Protonotariou E, Poulou A, Politi L, Sgouropoulos I, Metallidis S, Kachrimanidou M, et al. Hospital outbreak due to a *Klebsiella pneumoniae* ST147 clonal strain co-producing KPC-2 and VIM-1 carbapenemases in a tertiary teaching hospital in Northern Greece. Int J Antimicrob Agents. 2018;52:331-7.

[18] Protonotariou E, Poulou A, Politi L, Meletis G, Chatzopoulou F, Malousi A, et al. Clonal outbreak caused by VIM-4-producing *Proteus mirabilis* in a Greek tertiary-care hospital. Int J Antimicrob Agents. 2020;56:106060.

[19] Peirano G, Matsumura Y, Adams MD, Bradford P, Motyl M, Chen L, et al. Genomic Epidemiology of Global Carbapenemase-Producing *Enterobacter* spp., 2008-2014. Emerg Infect Dis. 2018;24:1010-9.

[20] Su HR, Turhan O, Erman Daloglu CA, Ogunc MD, Ozhak B, Ongut G, et al. Molecular Epidemiology of Carbapenem-Resistant Enterobacterales Strains Isolated from Blood Cultures in Antalya, Turkey. Lab Med. 2020;51:601-5.

LEGEND TO THE FIGURES

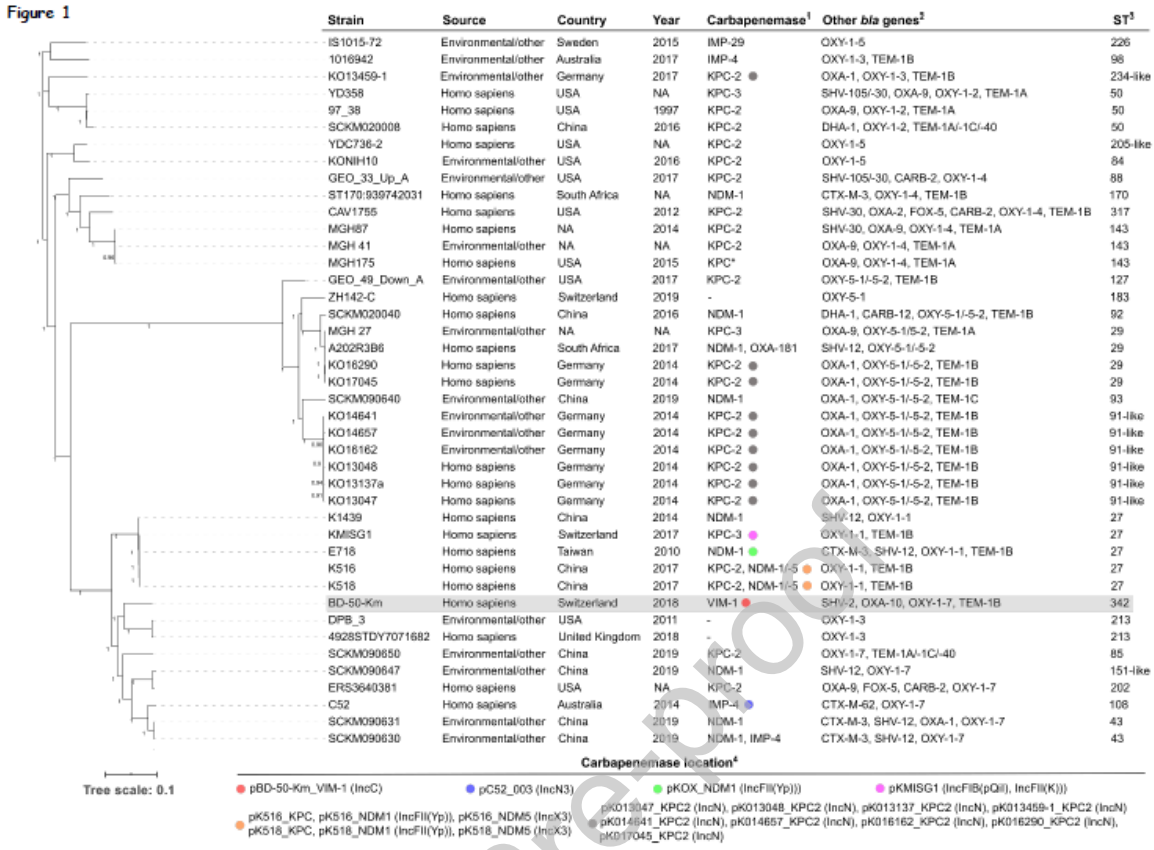

Figure 1. Core-genome analysis of 39 carbapenemase-producing *K. michiganensis* strains. The core-genome alignment and phylogeny were generated with Parsnp (v1.2) and resulted in 178'921 SNVs and a 64% core-genome alignment across all strains, which included the strain BD-50-Km as reference genome, and three non-carbapenemase producers (one Swiss stain and two closely-related strains). The tree was visualized with iTOL (v5.6.3; itol.embl.de). The tree scale represents the average number of nucleotide substitutions per site. SH-like support values are shown in the tree branches. Five IncC replicon sequences were detected in strains 97_38, CAV1755, GEO_33_Up_A, YD358, and ERS3640381 (Figure S1). NA; Not Available.

[1, 2] Carbapenemase and other *bla* genes were detected by ResFinder (v4.1). A gene name followed by a dash "/" corresponds to multiple or partial hits of the same gene family. For

strain MGH175, the KPC partial sequence was detected at 40% minimum length with ResFinder.

[3] MLST (v2.0) was performed for all strains according to the *K. oxytoca* scheme. Strains with a nearest ST are represented by a ST-like.

[4] The carbapenemase location is shown only when the gene is explicitly stated in the genome assembly as a plasmid or chromosomal sequence; its location is represented by a colored circle, plasmid name, and replicon type.

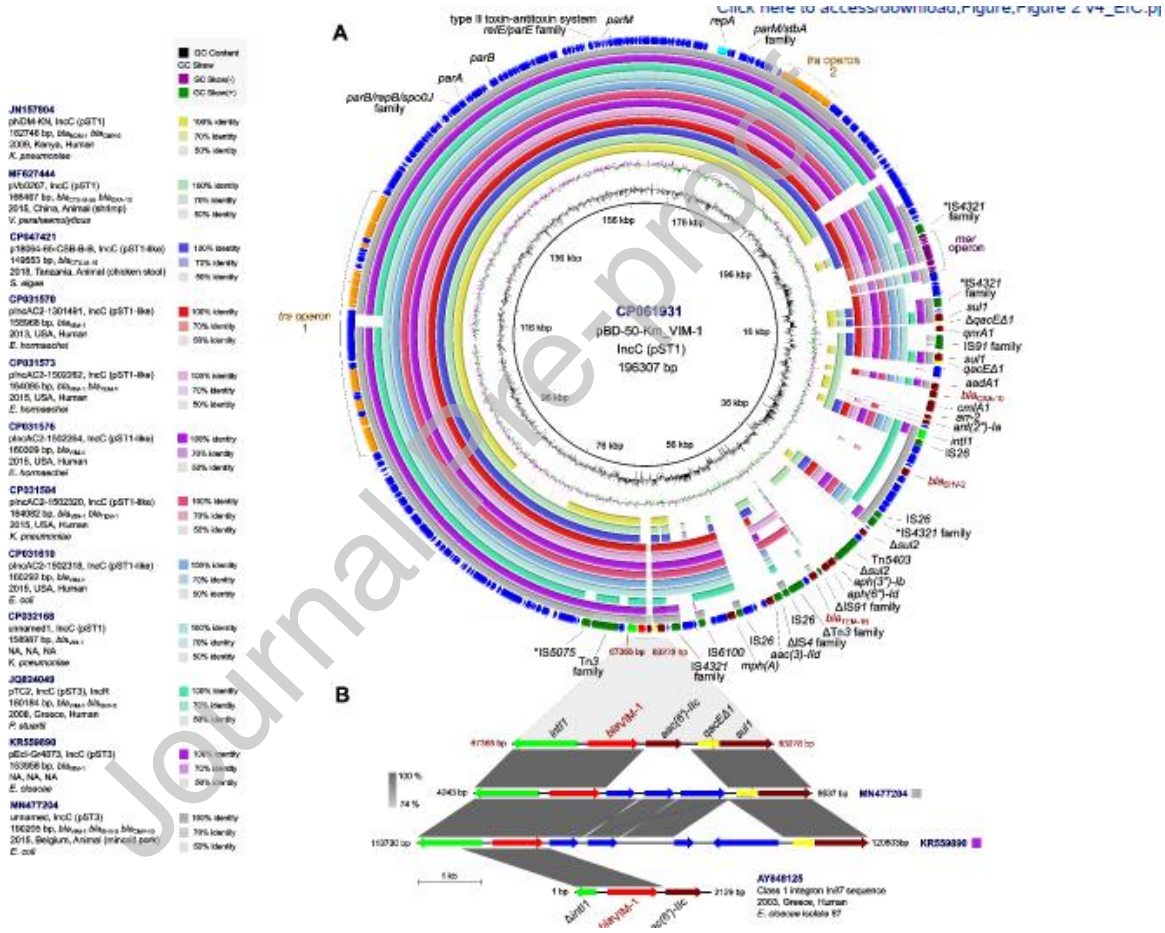

Figure 2. Circular BLAST comparison of pBD-50-Km_VIM-1 to other similar plasmid backbones and the integron region of *bla*$_{\text{VIM-1}}$.

(**A**) Comparison of the sequences of twelve multispecies IncC carrying plasmids. Rings were constructed using BRIG (BLAST Ring Image Generator; v0.95). Similarities with pBD-50-Km_VIM-1 are represented by the colored rings. GenBank accession numbers, plasmid name, replicon type and pMLST, plasmid size, main *bla*, year, country, source, and species are

indicated in the legend. Annotations above the rings correspond to the gene features of interest. The annotations for the *tra* operons 1 and 2, and *repA* are shown according to the nucleotide sequence alignment to the reference IncC plasmid pNDM-KN (JN157804). A delta (Δ) symbol before the gene name/feature corresponds to a partial/incomplete CDS; an asterisk (*) corresponds to a frameshifted product.

(**B**) Linear comparison of the $bla_{\text{VIM-1}}$ integron region against two plasmids with the most similar integron regions (MN477204 and KR559890), and the full length (2129bp) of the In*87* reference sequence (AY648125). The start (63278bp) and end (67368bp) positions of the integron are shown in red. The linear BLAST comparison was generated with Easyfig (v2.2.2). BLAST similarity is represented by the grey area in between the sequence alignment. Lines represent BLAST hits.

Table 1. Phenotypic characterization of the VIM-1-producing *K. michiganensis* BD-50-Km

Antibiotics	**MIC values (mg/L)** [a]
	***K. michiganensis* BD-50-Km** [a]
Piperacillin-tazobactam	>64/4 (R)
Ticarcillin-clavulanate	>128/2 (R)
Cefotaxime	32 (R)
Cefotaxime-clavulanate	32 (NA)
Ceftazidime	>128 (R)
Ceftazidime-clavulanate	>128 (NA)
Cefepime	8 (R)
Aztreonam	<2 (S)
Imipenem	2 (S)
Meropenem	<1 (S)
Doripenem	0.5 (NA)
Ertapenem	0.5 (S)
Gentamicin	>16 (R)
Tobramycin	4 (R)
Amikacin	<4 (S)

Ciprofloxacin	<0.25 (S)
Levofloxacin	<1 (S)
Doxycycline	<2 (NA)
Minocycline	<2 (NA)
Tigecycline	<0.25 (NA)
Trimethoprim/sulfamethoxazole	<0.5/9.5 (S)
Colistin	<0.25 (S)
Polymyxin B	<0.25 (NA)

Note. R, resistant; I, intermediate; S, susceptible; NA, not available; -, not tested

[a] MICs were obtained with microdilution Sensititre panels ESBF and GNX2F and interpreted according to the EUCAST 2019 criteria (version 9.0) for *Enterobacterales*.